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MASS-SPECTROMETRIC STUDY OF CARBOHYDRATES

METHYL ETHERS OF MONOSACCHARIDES

In recent years, for solving the question of the structure of complex organic compounds, especially natural compounds, the method of mass-spectrometric fragmentation analysis has been increasingly widely applied ([1,2]). This method has been used successfully in the chemistry of steroids ([3]), terpenoids ([4]), alkaloids ([5]), amino acids and peptides ([6]), and lipids ([7]). At the same time, in the study of carbohydrates, because of the low volatility of these compounds and certain features of their structure, the mass-spectrometric method has not been developed. The only data reported by Reed and co-workers concern measurements of the appearance potentials of α- and β-glycosides ([8]) and mass spectrometry of the methylated polysaccharide laminarin ([9]). On the basis of these data it is impossible to draw any conclusions about the applicability of the method to problems of the structural chemistry of carbohydrates. In the present work we give the first information on the mass spectra of carbohydrate derivatives, indicating the fundamental possibility of using mass spectrometry in the study of this most important class of natural compounds.

Our work was begun with the mass spectrometry of methyl ethers of monosaccharides, which were chosen for two reasons. As is known, it is precisely the structure of methylated monosaccharides that is especially often the object of study, since methylation and investigation of the hydrolysis products of methylated oligo- and polysaccharides underlie one of the principal methods for establishing the structure of these compounds. Identification of methyl ethers of monosaccharides by ordinary methods is extremely laborious and requires considerable amounts of substance. On the other hand, methyl ethers of monosaccharides are rather volatile compounds, which removes one of the main difficulties in the mass-spectrometric study of carbohydrates; at the same time, methylation disturbs the geometry of

Fig. 1

Figure 1: Fig. 1

the carbohydrate molecule to the least extent, and therefore, in studying methyl ethers, one may hope to obtain at least initial, guiding ideas about the pathways of fragmentation of the complex monosaccharide molecule. We recorded the mass spectra of 2,3,4,6-tetramethyl-*D*-glucopyranose (I), 2,3,5,6-tetramethyl-*D*-glucofuranose (II), 2,3,4,6-tetramethyl-*D*-galactopyranose (III), 2,3,4-trimethyl-*D*-xylopyranose (IV), 2,3,4-trimethyl-*L*-rhamnopyranose (V), and 2,3,5-trimethyl-*L*-arabofuranose (VI), which were obtained and purified to a chromatographically homogeneous state by conventional methods.

The mass spectra of these compounds, shown in Figs. 1 and 2, were recorded on an MX-1303 instrument equipped with a heated inlet system made entirely of stainless steel. The inlet system was maintained at a temperature of 175°. A sample weighing 1–2 mg was placed in a glass ampoule, which was evacuated, sealed, and placed in a vacuum lock; the ampoule was then broken, and the vapors of the sample passed into the inlet bulb, and from it through a molecular leak into the ionization chamber of the mass spectrometer. The spectrum was recorded with an EPP-09 electronic potentiometer. Ratios m/e above 200 were determined with an accuracy of ± 2.

The mass spectrum obtained was processed in the following way: the principal (highest) line of the spectrum was taken as 100%, and all the remaining lines were recalculated as percentages relative to it. Repeated measurements after such recalculation show good reproducibility of the results.

Fig. 1

As can be seen from the figures, the mass spectra of the compounds studied are externally constructed according to the same type. Each of them breaks down into two ranges: one (m/e from 230 approximately to 130) contains a small number of peaks of low intensity, while in the other (m/e from 130 to 15) there is a much larger number of peaks of high intensity. The peak corresponding to the molecular ion, as a rule, cannot be observed. The most intense peaks in spectra I, III, and IV are those corresponding to masses 101, 88, and 45. Peaks with small masses (15, 17, 18), which are sufficiently intense and correspond to small fragments of the molecule (methyl and hydroxyl groups, etc.), are of little interest and were not taken into account by us.

Despite the external similarity, the mass spectra of the substances studied contain a number of details that make it possible to distinguish them clearly from one another. This fact in itself is of considerable significance, since, taking into account the good reproducibility of the spectra, it opens up the possibility of developing a new method for identifying methylated monosaccharides requiring a minimal amount of the substance under study.

The spectra of methylated hexapyranoses I and III, despite their external similarity, differ from one another in that in spectrum I the peaks 88 and 45 amount respectively to 65 and 63% of peak 101, whereas in spectrum III peak 45 has an intensity of 39, and peak 88—44%. The spectrum of 2,3,4-trimethylxylopyranose (IV), which is structurally close to these substances, is clearly distinguished from spectra I and III by the absence of peaks 191 and 218 and by a different ratio of the intensities of the peaks with masses 45 and 88, which for I and III are close in intensity, while for IV the peak with mass 45 is 10% smaller than the peak with mass 88. The spectrum of 2,3,4-trimethylrhamnose (V) differs from spectra I, III, and IV by a significant increase in the intensity of the peaks 43, 75, and 88. These data show that the mass spectra of aldopyranoses of different types—hexapyranoses, pentapyranoses, and methylpentapyranoses—have their own characteristic features, making it possible to distinguish one type from another.

The spectra of methylated furanoses (II and VI) differ no less sharply from one another. In spectrum VI the principal peaks are 45 and 101, followed by 87 (91%) and 115 (83%). In spectrum II the principal peak corresponds to mass 89; peaks 45 (91%), 101 (85%), 141 (82%), 87 (79%), and 59 (66%) have somewhat lower intensity. Comparison of these spectra with the spectra of pyranoses discussed above shows that characteristic differences exist between them.

Consideration of the spectra of compounds I—VI permits preliminary conclusions to be drawn about the pathways of decomposition of the molecules of methylated monosaccharides; this may be of considerable importance for judging the pathways of fragmentation of carbohydrate molecules in general and, consequently, for the whole question of the further use of the method. Let us examine in greater detail the spectrum of glucopyranose I and, using it as an example, try to derive certain regularities. It is evident that decomposition begins with loss of a molecule of water (18) and formation of a fragment of mass 218. Another readily eliminated fragment is the side chain—CH_2OCH_3 (45), loss of which leads to fragments with masses 191 or 173 (236—18—45). Similarly, i.e., with elimination of water from one side and of the side chain from the other side, decomposition of the furanose derivative II also begins; in its spectrum the most intense peak, 89, may be assigned to the side-chain fragment $CH(OCH_3)CH_2(OCH_3)$, while peak 141 to the residue of the molecule after loss of water, C_3HOH and $-CH_2OCH_3$ (236—18—32—45). At the same time it is somewhat more difficult to make judgments about the further decomposition of the large fragments remaining after loss of water, methanol, and the side chain. One of the most intense peaks in all spectra—the peak 88—probably belongs to the fragment $-CH(OCH_3)-CH(OCH_3)$, and peak 101 to $>CH-CH(OCH_3)CH(OCH_3)$. Thus, the impression is created that under the action of electron impact the monosaccharide molecule breaks in half with formation of C_2- and C_3-fragments, which involuntarily brings to mind certain metabolic pathways of carbohydrate decomposition.

Fig. 2

Fig. 2

Figure 2: Fig. 2

These assumptions make it possible to explain the similarity of spectra I, III, and IV and some features of spectra II, V, and VI. For example, the increase in the intensity of peak 88 in spectrum V can be explained by the fact that this mass is possessed by two fragments: $-CH(OCH_3)CH(OCH_3)$ and $CH_3CH(O-)CH \cdot (OCH_3)-$.

Naturally, the considerations concerning the pathways of decomposition of carbohydrate molecules are as yet of the most preliminary character and will probably be substantially refined as new experimental material accumulates. At the same time, the considerations set forth make it possible to establish certain starting points necessary for the further development of the study.

The factual material presented in this article and the conclusions drawn from it concerning the characteristic features of spectra of one or another type of carbohydrate are subject to further clarification, expansion, and critical evaluation. However, the data presented undoubtedly show that the mass-spectrometric analysis of carbohydrate derivatives, described for the first time in this article, has serious prospects and deserves much attention. The same is indicated by the data concerning other carbohydrate derivatives at our disposal, which will be published later.

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Note: Figure translations are in progress. See original paper for figures.